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(54) Title: LIPOLYTIC ENZYME GENES

(57) Abstract: The inventors have isolated novel genes with a high homology to the *T. lanuginosus* lipase gene and are thus well suited for use in gene shuffling. Accordingly, the invention provides a method of generating genetic diversity into lipolytic enzymes by family shuffling of two or more homologous genes which encode lipolytic enzymes. The DNA shuffling technique is used to create a library of shuffled genes, and this is expressed in a suitable expression system and the expressed proteins are screened for lipolytic enzyme activity. The expressed proteins may further be screened to identify lipolytic enzymes with improved properties. The invention also provides a polynucleotide comprising a nucleotide sequence encoding a lipolytic enzyme and a lipolytic enzyme (a polypeptide with lipolytic enzyme activity).

LIPOLYTIC ENZYME GENES

FIELD OF THE INVENTION

The present invention relates to a method of generating diversity into lipolytic enzymes by the use of the so-called family shuffling of homologous genes. The invention also relates to polynucleotides for use in the method, and to lipolytic enzymes encoded by the polynucleotides.

BACKGROUND OF THE INVENTION

The lipase of *Thermomyces lanuginosus* (also known as *Humicola lanuginosa*) is known to be useful for various industrial purposes such as detergents and baking (EP 258068, WO 9404035). Its amino acid and DNA sequences are shown in US 5869438.

The prior art describes the modification of the amino acid sequence of the *T. lanuginosus* lipase to create variants with the aim of modifying the enzyme properties. Thus, US 5869438, WO 9522615, WO 9704079 and WO 0032758 disclose the use of mutagenesis of the lipase gene to produce such variants. WO 0032758 also discloses the construction of variants with the backbone from *T. lanuginosus* lipase and C-terminal from *Fusarium oxysporum* phospholipase by PCR reaction.

Crameri et al, 1998, Nature, 391: 288-291 discloses DNA shuffling of a family of naturally occurring homologous genes from diverse species to create diversity into proteins. US 6159687 discloses shuffling of genes encoding variants of the *T. lanuginosus* lipase. WO 9841623 discloses shuffling of heterologous polynucleotide sequences.

The following published sequences of lipolytic enzymes from *Aspergillus* have amino acid identities of 49-51 % to the *T. lanuginosus* lipase: Lysophospholipase from *A. foetidus* (EMBL A93428, US 6140094), lipase from *A. tubingensis* (EMBL A84589, WO 9845453), phospholipase A1 from *A. oryzae* (EMBL E16314, EP 575133, JP 10155493 A) and Lysophospholipase from *A. niger* (EMBL A90761, WO 98/31790).

R. Lattmann et al., Biocatalysis, 3 (1-2), 137-144 (1990) disclose an esterase from *Talaromyces thermophilus*. V.W. Ogundero, Mycologia, 72 (1), 118-126 (1980) describes the lipase activity of *Talaromyces thermophilus*. US 4275011 and EP 258068 refer to a lipase from *Thermomyces ibadanensis*. B.A. Oso, Canadian Journal of Botany, 56: 1840-1843 (1978) describes the lipase activity of *Talaromyces emersonii*.

SUMMARY OF THE INVENTION

The inventors have isolated novel lipolytic enzyme genes with a high homology to the *T. lanuginosus* lipase gene and are thus well suited for use in gene shuffling. The novel genes are shown as SEQ ID NO: 3, 5, 7, 9 and 11. Identity tables for some protein and DNA sequences are shown below. The novel sequences are identified as follows:

- Talthe1M: SEQ ID NO: 3 and 4 from *Talaromyces thermophilus.*
- Theiba1M: SEQ ID NO: 5 and 6 from *Thermomyces ibadanensis.*
- Taleme1M: SEQ ID NO: 7 and 8 from *Talaromyces emersonii.*
- Talbys1M: SEQ ID NO: 9 and 10 from *Talaromyces byssochlamydoides.*

The following known sequences are included for comparison:

- Thelan1M: Lipase from *Thermomyces lanuginosus*, SEQ ID NO: 1 and 2.
- Asptub2M: EMBL A84589 Lipase from *Aspergillus tubingensis.*
- Aspory3M: EMBL E16314 Phospholipase A1 from *Aspergillus oryzae.*
- Aspnig2M: EMBL A90761 Lysophospholipase from *Aspergillus niger.*

The following is an identity table of the mature proteins:

	Thelan1	Talthe1	Theiba1	Taleme1	Talbys1	Asptub2	Aspory3	Aspnig2
Thelan1M	100.0	88.1	78.1	61.9	57.4	50.6	50.4	49.1
Talthe1M	88.1	100.0	78.8	61.5	59.2	48.7	47.8	48.0
Theiba1M	78.1	78.8	100.0	61.8	58.0	49.4	50.4	48.0
Taleme1M	61.9	61.5	61.8	100.0	83.1	54.8	56.1	53.7
Talbys1M	57.4	59.2	58.0	83.1	100.0	50.9	54.9	49.1
Asptub2M	50.6	48.7	49.4	54.8	50.9	100.0	55.9	93.7
Aspory3M	50.4	47.8	50.4	56.1	54.9	55.9	100.0	53.7
Aspnig2M	49.1	48.0	48.0	53.7	49.1	93.7	53.7	100.0

The following is an identity table of DNA sequences coding for the mature proteins (stop codons omitted):

	Thelan1	Talthe1	Theiba1	Taleme1	Talbys1	Asptub2	Aspory3	Aspnig2
Thelan1M	100.0	86.0	79.3	62.0	58.4	57.0	55.6	56.2
Talthe1M	86.0	100.0	79.1	62.6	60.0	57.8	55.7	57.1
Theiba1M	79.3	79.1	100.0	63.5	60.4	56.6	57.8	55.6
Taleme1M	62.0	62.6	63.5	100.0	84.1	58.2	58.4	58.7
Talbys1M	58.4	60.0	60.4	84.1	100.0	57.5	56.5	56.8
Asptub2M	57.0	57.8	56.6	58.2	57.5	100.0	58.7	91.7

Aspory3M	55.6	55.7	57.8	58.4	56.5	58.7	100.0	56.5
Aspnig2M	56.2	57.1	55.6	58.7	56.8	91.7	56.5	100.0

Accordingly, the invention provides a method of generating genetic diversity into lipolytic enzymes by family shuffling of two or more homologous genes which encode lipolytic enzymes. One gene encodes a lipolytic enzyme with at least 90 % identity to the *T. lanuginosus* lipase, and another gene encodes a lipolytic enzyme with 55-90 % identity to the *T. lanuginosus* lipase. The DNA shuffling technique is used to create a library of chimeric shuffled genes, and this is expressed in a suitable expression system and the expressed proteins are screened for lipolytic enzyme activity. The expressed proteins may further be screened to identify lipolytic enzymes with improved properties.

The invention also provides a polynucleotide comprising a nucleotide sequence encoding a lipolytic enzyme and a lipolytic enzyme (a polypeptide with lipolytic enzyme activity).

The polynucleotide may be a DNA sequence cloned into a plasmid present in *E. coli* deposit number DSM 14047, 14048, 14049, or 14051, the DNA sequence encoding a mature peptide shown in SEQ ID NO: 3, 5, 7 or 9 or one that can be derived therefrom by substitution, deletion, and/or insertion of one or more nucleotides. The polynucleotide may have at least 90 % identity with the DNA sequence encoding a mature peptide shown in SEQ ID NO: 3, at least 80 % identity with the DNA sequence encoding a mature peptide shown in SEQ ID NO: 5, at least 65 % identity with the DNA sequence encoding a mature peptide shown in SEQ ID NO: 7, or at least 60 % identity with the DNA sequence encoding a mature peptide shown in SEQ ID NO: 9. It may also be an allelic variant of the DNA sequence encoding a mature peptide shown in SEQ ID NO: 3, 5, 7 or 9; or it may hybridize under high stringency conditions with a complementary strand of the nucleic acid sequence encoding a mature peptide shown in SEQ ID NO: 3, 5, 7 or 9, or a subsequence thereof having at least 100 nucleotides.

The lipolytic enzyme may be encoded by a DNA sequence cloned into a plasmid present in *E. coli* deposit number DSM 14047 or 14049, or may have an amino acid sequence which is the mature peptide of SEQ ID NO: 6 or 10, or one that can be derived therefrom by substitution, deletion, and/or insertion of one or more amino acids. The lipolytic enzyme may have an amino acid sequence which has at least 80 % identity with the mature peptide of SEQ ID NO: 6 or at least 60 % identity with the mature peptide of SEQ ID NO: 10. The lipolytic enzyme may further be immunologically reactive with an antibody raised against the mature peptide of SEQ ID NO: 6 or 10 in purified form, be an allelic variant of the mature peptide of SEQ ID NO: 6 or 10; or be encoded by a nucleic acid sequence which hybridizes under high strin-

gency conditions with a complementary strand of the nucleic acid sequence encoding a mature peptide shown in SEQ ID NO: 5 or 9, or a subsequence thereof having at least 100 nucleotides.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 shows a PCR scheme used in Example 7.

DETAILED DESCRIPTION OF THE INVENTION

Genomic DNA source

Lipolytic enzyme genes of the invention may be derived from strains of *Talaromyces* or *Thermomyces*, particularly *Talaromyces thermophilus*, *Thermomyces ibadanensis*, *Talaromyces emersonii* or *Talaromyces byssochlamydoides*, using probes designed on the basis of the DNA sequences in this specification.

Thus, genes and polypeptides shown in the sequence listing were isolated from the organisms indicated below. Strains of *Escherichia coli* containing the genes were deposited by the inventors under the terms of the Budapest Treaty with the DSMZ - Deutsche Sammlung von Microorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig DE as follows:

Source organism	Gene and polypeptide sequences	Clone deposit No.	Clone deposit date
Talaromyces thermophilus ATCC 10518	SEQ ID NO: 3 and 4	DSM 14051	8 February 2001
Thermomyces ibadanensis CBS 281.67 = ATCC 22716	SEQ ID NO: 5 and 6	DSM 14049	8 February 2001
Talaromyces emersonii UAMH 5005= NRRL 3221 = ATCC 16479 = IMI 116815 = CBS 393.64	SEQ ID NO: 7 and 8	DSM 14048	8 February 2001
Talaromyces byssochlamydoides CBS 413.71 = IMI 178524 = NRRL 3658	SEQ ID NO: 9 and 10	DSM 14047	8 February 2001

The above source organisms are freely available on commercial terms from the following strain collections:

ATCC (American Type Culture Collection), 10801 University Boulevard, Manassas, VA 20110-2209, USA.

CBS (Centraalbureau voor Schimmelcultures), Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.

UAMH (University of Alberta Mold Herbarium & Culture Collection), Devonian Botanic Garden, Edmonton, Alberta, Canada T6G 3GI.

IMI: International Mycological Institute, Bakeham Lane, Englefield Green, EGHAM, Surrey TW20 9TY, United Kingdom.

Polynucleotides

The polynucleotides to be used for recombination (shuffling) are two or more genes encoding lipolytic enzymes, including one with at least 90 % identity and one with 55-90 % identity to the *T. lanuginosus* lipase (SEQ ID NO: 2). The poloynucleotides differ in at least one nucleotide.

The starting material may include the mature part of two or more (e.g. three, four or five) of SEQ ID NO: 1, 3, 5, 7 and/or 9. It may also include genes encoding two or more (e.g. three, four or five) of variants of SEQ ID NO: 2, 4, 6, 8 or 10 obtained by deleting, substituting and/or inserting one or more amino acids and/or by attaching a peptide extension at the N- and/or C-terminal. Examples of variants of the *T. lanuginosus* lipase are described, e.g., in US 5869438, WO 9522615, WO 9704079 and WO 0032758, and similar variants can be made by altering corresponding amino acids in the other sequences.

Any introns present in the genes may optionally be removed before the shuffling.

DNA recombination (shuffling)

Shuffling between two or more homologous input polynucleotides (starting-point polynucleotides) may involve fragmenting the polynucleotides and recombining the fragments, to obtain output polynucleotides (i.e. polynucleotides that have been subjected to a shuffling cycle) wherein a number of nucleotide fragments are exchanged in comparison to the input polynucleotides.

DNA recombination or shuffling may be a (partially) random process in which a library of chimeric genes is generated from two or more starting genes. A number of known formats can be used to carry out this shuffling or recombination process.

The process may involve random fragmentation of parental DNA followed by reassembly by PCR to new full length genes, e.g. as presented in US5605793, US5811238, US5830721, US6117679 . In-vitro recombination of genes may be carried out, e.g. as described in US6159687, WO98/41623, US6159688, US5965408, US6153510. The recombination process may take place *in vivo* in a living cell, e.g. as described in WO 97/07205 and WO 98/28416.

The parental DNA may be fragmented by DNA'se I treatment or by restriction endonuclease digests as descriobed by Kikuchi et al (2000a , Gene 236:159-167). Shuffling of two parents may be done by shuffling single stranded parental DNA of the two parents as described in Kikuchi et al (2000b , Gene 243:133-137).

A particular method of shuffling is to follow the methods described in Crameri et al, 1998, Nature, 391: 288-291 and Ness et al. Nature Biotechnology 17: 893-896. Another format would be the methods described in US 6159687: example 1 and 2.

Properties of lipolytic enzyme

The lipolytic enzyme obtained by the invention is able to hydrolyze carboxylic ester bonds and is classified as EC 3.1.1 according to Enzyme Nomenclature 1992, Academic Press, Inc. It may particularly have activity as a lipase (triacylglycerol lipase) (EC 3.1.1.3), phospholipase A1 (EC 3.1.1.32), phospholipase A2 (EC 3.1.1.4), cholesterol esterase (EC 3.1.1.13) and/or galactolipase (EC 3.1.1.26).

The thermostability was evaluated by means of Differential Scanning Calorimetry (DSC). The denaturation peak (T_d) when heated at 90 deg/hr at pH 5 is slightly above 75°C for the lipolytic enzyme from *T. ibadanensis*, compared to slightly above 70 °C for the prior-art *T. lanuginosus* lipase. The lipolytic enzyme from *T. ibadanensis* has optimum activity at alkaline pH (similar to the *T. lanuginosus* lipase) and has an isoelectric point of about 4.3 (slightly lower than the *T. lanuginosus* lipase).

Homology and alignment

The best alignment of the mature parts of SEQ ID NO: 2, 4, 6, 8 and 10 is achieved by inserting a gap of one amino acid between Q249 and P/G250 of SEQ ID NO: 2, 4 and 6. This alignment defines corresponding amino acids.

The degree of homology may be determined by means of computer programs known in the art, such as GAP provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711) (Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48, 443-45), using GAP with the following settings for polypeptide sequence comparison: GAP creation penalty of 3.0 and GAP extension penalty of 0.1.

The determination of homology may also be made using Align from the fasta package version v20u6. Align is a Needleman-Wunsch alignment (i.e. global alignment), useful for both protein and DNA alignments. The default scoring matrices BLOSUM50 and the identity matrix are used for protein and DNA alignments respectively. The penalty for the first residue in a gap

is -12 for proteins and -16 for DNA. While the penalty for additional residues in a gap is -2 for proteins and -4 for DNA.

The homologies discussed in this specification may correspond to at least 60 % identity, in particular to at least 70 % or at least 80 % identity, e.g. at least 90 % or at least 95 % identity.

Use of lipolytic enzyme

Depending on the substrate specificity, the enzyme of the invention can be used, e.g., in filtration improvement, vegetable oil treatment, baking, detergents, or preparation of lysophospholipid. Thus, it may be used in known applications of lipolytic enzymes by analogy with the prior art, e.g.:

- In the pulp and paper industry, to remove pitch or to remove ink from used paper. WO 9213130, WO 9207138, JP 2160984 A, EP 374700.
- Baking. WO 94/04035, WO 00/32758.
- Detergents. WO 97/04079, WO 97/07202, WO 97/41212, WO 98/08939 and WO 97/43375.
- Leather industry. GB 2233665, EP 505920.
- An enzyme with lipase activity may be used for fat hydrolysis and for modification of triglycerides and for production of mono- and diglycerides.
- An enzyme with lipase activity may be used for interesterification of bulk fats, production of frying fats, shortenings and margarine components.
- An enzyme with phospholipase activity (A1, A2) may be used for degumming of vegetable oils and for lysophospholipid production.

Improvement of filtration

An enzyme with lysophospholipase activity can be used to improve the filterability of an aqueous solution or slurry of carbohydrate origin by treating it with the variant. This is particularly applicable to a solution or slurry containing a starch hydrolysate, especially a wheat starch hydrolysate since this tends to be difficult to filter and to give cloudy filtrates. The treatment can be done in analogy with EP 219,269 (CPC International).

Detergents

The lipolytic enzyme produced by the invention may be used as a detergent additive, e.g. at a concentration (expressed as pure enzyme protein) of 0.001-10 (e.g. 0.01-1) mg per gram of detergent or 0.001-100 (e.g. 0.01-10) mg per liter of wash liquor.

The detergent composition of the invention may for example be formulated as a hand or machine laundry detergent composition including a laundry additive composition suitable for pre-treatment of stained fabrics and a rinse added fabric softener composition, or be formulated as a detergent composition for use in general household hard surface cleaning operations. In a laundry detergent, the variant may be effective for the removal of fatty stains, for whiteness maintenance and for dingy cleanup. A laundry detergent composition may be formulated as described in WO 97/04079, WO 97/07202, WO 97/41212, PCT/DK WO 98/08939 and WO 97/43375.

The detergent composition of the invention may particularly be formulated for hand or machine dishwashing operations. e.g. as described in GB 2,247,025 (Unilever) or WO 99/01531 (Procter & Gamble). In a dishwashing composition, the variant may be effective for removal of greasy/oily stains, for prevention of the staining /discoloration of the dishware and plastic components of the dishwasher by highly colored components and the avoidance of lime soap deposits on the dishware.

The detergent composition of the invention may be in any convenient form, e.g., a bar, a tablet, a powder, a granule, a paste or a liquid. A liquid detergent may be aqueous, typically containing up to 70 % water and 0-30 % organic solvent, or non-aqueous.

The detergent composition comprises one or more surfactants, which may be non-ionic including semi-polar and/or anionic and/or cationic and/or zwitterionic. The surfactants are typically present at a level of from 0.1% to 60% by weight, e.g. 0.5-40 %, such as 1-30 %, typically 1.5-20 %.

Dough and baked products

The lipolytic enzyme can be used in the preparation of dough and baked products made from dough, such as bread and cakes, e.g. to increase dough stability and dough handling properties, or to improve the elasticity of the bread or cake. Thus, it can be used in a process for making bread, comprising adding it to the ingredients of a dough, kneading the dough and baking the dough to make the bread. This can be done in analogy with US 4,567,046 (Kyowa Hakko), JP-A 60-78529 (QP Corp.), JP-A 62-111629 (QP Corp.), JP-A 63-258528 (QP Corp.) or EP 426211 (Unilever). The lipolytic enzyme may be used together with an anti-staling amylase, particularly an endo-amylase such as a maltogenic amylase in analogy with WO 99/53769 (Novo Nordisk). Thus, the lipolytic enzyme may be incorporated in a flour composition such as a dough or a premix for dough.

MATERIALS AND METHODS

Strains and plasmids:

Plasmid pMT2188

The *Aspergillus oryzae* expression plasmid pCaHj 483 (WO 98/00529) consists of an expression cassette based on the *Aspergillus niger* neutral amylase II promoter fused to the *Aspergillus nidulans* triose phosphate isomerase non translated leader sequence (Pna2/tpi) and the *A. niger* amyloglycosidase terminator (Tamg). Also present on the plasmid is the *Aspergillus* selective marker *amdS* from *A. nidulans* enabling growth on acetamide as sole nitrogen source. These elements are cloned into the *E. coli* vector pUC19 (New England Biolabs). The ampicillin resistance marker enabling selection in *E. coli* of this plasmid was replaced with the URA3 marker of *Saccharomyces cerevisiae* that can complement a *pyrF* mutation in *E. coli*, the replacement was done in the following way:

The pUC19 origin of replication was PCR amplified from pCaHj483 with the primers 142779 (SEQ ID NO: 35) and 142780 (SEQ ID NO: 36).

Primer 142780 introduces a *Bbu*I site in the PCR fragment. The Expand PCR system (Roche Molecular Biochemicals, Basel, Switserland) was used for the amplification following the manufacturers instructions for this and the subsequent PCR amplifications.

The URA3 gene was amplified from the general *S. cerevisiae* cloning vector pYES2 (Invitrogen corporation, Carlsbad, Ca, USA) using the primers 140288 (SEQ ID NO: 37) and 142778 (SEQ ID NO: 38).

Primer 140288 introduces an *Eco*RI site in the PCR fragment. The two PCR fragments were fused by mixing them and amplifying using the primers 142780 and 140288 in the splicing by overlap method (Horton et al (1989) Gene, 77, 61-68).

The resulting fragment was digested with *Eco*RI and *Bbu*I and ligated to the largest fragment of pCaHj 483 digested with the same enzymes. The ligation mixture was used to transform the *pyrF*⁻ *E.coli* strain DB6507 (ATCC 35673) made competent by the method of Mandel and Higa (Mandel, M. and A. Higa (1970) J. Mol. Biol. 45, 154). Transformants were selected on solid M9 medium (Sambrook et. al (1989) Molecular cloning, a laboratory manual, 2. edition, Cold Spring Harbor Laboratory Press) supplemented with 1 g/l casaminoacids, 500 µg/l thiamine and 10 mg/l kanamycin.

A plasmid from a selected transformant was termed pCaHj 527. ThePna2/tpi promoter present on pCaHj527 was subjected to site directed mutagenises by a simple PCR approach.

Nucleotide 134 – 144 was altered from SEQ ID NO: 39 to SEQ ID NO: 40 using the mutagenic primer 141223 (SEQ ID NO: 41).

Nucleotide 423 – 436 was altered from SEQ ID NO: 42 to SEQ ID NO: 43 using the mutagenic primer 141222 (SEQ ID 44).

The resulting plasmid was termed pMT2188.

Plasmid pENI1861

Plasmid pENI1861 was made in order to have the state of the art *Aspergillus* promoter in the expression plasmid, as well as a number of unique restriction sites for cloning.

A PCR fragment (app. 620 bp) was made using pMT2188 (see above) as template and the primers 051199J1 (SEQ ID 45) and 1298TAKA (SEQ ID 46).

The fragment was cut BssHII and Bgl II, and cloned into pENI1849 which was also cut with BssHII and Bgl II. The cloning was verified by sequencing. Plasmid pENI1902 was made in order to have a promoter that works in both *E.coli* and *Aspergillus*. This was done by unique site elimination using the "Chameleon double stranded site-directed mutagenesis kit" as recommended by Stratagene®.

Plasmid pENI1861

Plasmid pENI1861 was used as template and the following primers with 5` phosphorylation were used as selection primers: 177996 (SEQ ID 47), 135640 (SEQ ID 48) and 135638 (SEQ ID 49).

The 080399J19 primer (SEQ ID NO: 50) with 5` phosphorylation was used as mutagenic primer to introduce a –35 and –10 promoter consensus sequence (from *E.coli*) in the *Aspergillus* expression promoter. Introduction of the mutations was verified by sequencing.

Plasmid pENI1960

Plasmid pENI1960 was made using the Gateway Vector™ conversion system (Lifetechnology® cat no. 11828-019) by cutting pENI1902 with BamHI, filling the DNA ends using Klenow fragment polymerase and nucleotides (thus making blunt ends) followed by ligation to reading frame A Gateway™ PCR fragment. The cloning in the correct orientation was confirmed by sequencing.

Media and substrates

YPG: 4 g/L Yeast extract, 1 g/L KH2PO4, 0.5 g/L MgSO4-7aq, 5 g/L Glucose, pH 6.0.

EXAMPLES

Example 1: Plasmids harboring lipolytic enzyme genes

Genomic DNA preparation

Strains of *Thermomyces ibadanensis*, *Talaromyces emersonii*, *Talaromyces byssochlamydoides*, and *Talaromyces thermophilus* were used as a genomic DNA supplier. Each strain was cultivated in 100 ml of YPG at appropriate temperature for several days. Mycelia was harvested and ground in liquid N_2. It was suspended with 2 ml of 50 mM Tris-HCl (pH8.0) buffer including 100 mM NaCl, 25 mM EDTA, and 1% SDS and then 12µl of proteinase K (25 mg/ml) was added. The suspension was incubated at 65° C for 30~60min. Phenol extraction was done to remove proteins and DNA was precipitated by 0.7 volume of isopropanol. The precipitate was dissolved with sterilized water and RNase was added. After Phenol / isoamylalcohol extraction, DNA was precipitated by EtOH.

PCR screening of lipolytic enzyme genes

PCR reactions on each genomic DNA was done with HL 2 and HL12 (SEQ ID NO: 51 and 52) or HL2 and HL6 (SEQ ID NO: 51 and 53) designed based upon alignment lipases.

Reaction components (2.6 ng /µl of genomic DNA, 250 mM dNTP each, primer 250 nM each, 0.1 U/ µl of Taq polymerase in 1X buffer (Roche Diagnostics, Japan)) were mixed and submitted for PCR under the following conditions.

Step	Temperature	Time
1	94°C	1 min
3	50°C	1 min
4	72°C	2 min
5	72°C	10 min
6	4°C	forever

Steps 1 to 3 were repeated 30 times.

540 bp of fragment and 380 bp of fragment were amplified from primer sets of HL2/HL12 and HL2/HL6, respectively. They were gel-purified with GFX™ PCR DNA and Gel Band Purification kit (amersham pharmacia biotech) Each DNA was sequenced and compared to the lipase, showing that a clone encodes the internal part of the lipase.

Cloning of lipase genes

All lipase genes were cloned using LA PCR™ in vitro Cloning Kit (TaKaRa) according to the manufacturer's instructions. Thus, genomic DNA was cut with various restriction enzymes and each DNA was ligated with the appropriate cassette of the kit. Each ligation solution was applied to PCR with the primers of the one designed from internal sequence and a cassette primer of the kit. Amplified DAN fragment was sequenced. This step was repeated till ORF was determined.

The fidelity of LA- taq polymerase of the kit is not good so in order to get the right sequence whole gene was amplified by Expand high fidelity polymerase according to the manufacturer's instructions.

Amplified DNA fragment was gel-purified with GFX™ PCR DNA and Gel Band Purification kit (Amersham Pharmacia Biotech) and ligated into a pT7Blue vector or pST BLue -1 AccepTor vector (Novagen) with ligation high (TOYOBO, Japan) . The ligation mixtures were transformed into *E. coli* JM109 or DH5α. The sequence of four plasmids of each gene was determined and their sequence were compared. The sequence of majority is defined as the right nucleotide sequence.

Example 2: Cloning of lipase into *Aspergillus* expression vector.

3 different PCR reaction were run using PWO polymerase in the following reaction 94°C 5 min, 30* (94°C 30 sec., 50°C 30 sec, 72°C 2 min), 72°C 5min). In each case, the template was a plasmid harboring a lipolytic enzyme gene prepared as in Example 1, and the following primers were used:

A: Plasmid with gene from *Talaromyces thermophilus* and oligo 051200j1 /051200j8 (SEQ ID NO: 11 and 18).

B: Plasmid with gene from *Talaromyces emersonii* and oligo 051200j9 /051200j16 (SEQ ID NO: 19 and 26).

C: Plasmid with gene from *Thermomyces lbadanensis* and oligo 051200j17/051200j24 (SEQ ID NO: 27 and 34).

The PCR fragments were run and purified from a 1% agarose gel and cloned into pENI1960 (see above) using Gateway cloning as recommended by the supplier (Life Technologies) and transformed into *E.coli* DH10b (Life Technologies, Gaithersburg, MD) and sequenced, thus creating pENI 2146 (*Talaromyces emersonii* lipase gene), pENI2147 (*Thermomyces lbadanensis* lipase gene) and pENI2148 (*Talaromyces thermophilus* lipase gene).

These were transformed into Jal250 (described in WO 00/39322) and lipase activity identified as mentioned in pat WO 00/24883.

Example 3: Construction of intron-less lipase genes

Removal of introns from *Talaromyces thermophilus* lipase gene

4 PCR reactions were run using PWO polymerase and pENI2148 as template (94°C 5 min, 30* (94°C 30 sec., 50°C 30 sec, 72°C 1 min), 72°C 5min) and the following oligoes:

1: 051200j1 and 051200j3 (SEQ ID NO: 11 and 13)

2: 051200j2 and 051200j5 (SEQ ID NO: 12 and 15)

3: 051200j4 and 051200j7 (SEQ ID NO: 14 and 17)

4: 051200j6 and 051200j8 (SEQ ID NO: 16 and 18)

The specific bands were run and purified from a 1.5 % agarose gel. Equal amounts of PCR fragments were mixed along with PWO polymerase, buffer, dNTP, oligo 051200j1 and 051200j8 (SEQ ID NO: 11 and 18, total of 50 µl, as recommended by the supplier Boehringer Mannheim) and a second PCR was run (94°C 5 min, 30* (94°C 30 sec., 50°C 30 sec, 72°C 2 min), 72°C 5min).

The correct band size was checked on a 1.5 % agarose gel (app. 900 bp) and the rest of the PCR-fragment was purified using Biorad spin columns (cat no.732-6225)

The PCR-fragment was cloned into pENI1960 cut with ScaI (in order to cleave in the ccdB gene) using Gateway cloning as recommended by the supplier (Life Technologies) and transformed into *E. coli* DH10b and sequenced, thus creating intron-less *Talaromyces thermophilus* lipase gene.

Removal of introns from *Talaromyces emersonii* lipase gene

4 PCR reactions were run using PWO polymerase and pENI2146 as template (94°C 5 min, 30* (94°C 30 sec., 50°C 30 sec, 72°C 1 min), 72°C 5min) and the following oligoes:

1: 051200j9 and 051200j11 (SEQ ID NO: 19 and 21).

2: 051200j10 and 051200j13 (SEQ ID NO: 20 and 23).

3: 051200j12 and 051200j15 (SEQ ID NO: 22 and 25).

4: 051200j14 and 051200j16 (SEQ ID NO: 24 and 26).

The specific bands were run and purified from a 1.5 % agarose gel. Equal amounts of PCR fragments were mixed along with PWO polymerase, buffer, dNTP, oligo 051200j9 and 051200j16 (SEQ ID NO: 19 and 26, total of 50 µl, as recommended by the supplier) and a second PCR was run (94°C 5 min, 30* (94°C 30 sec., 50°C 30 sec, 72°C 2 min), 72°C 5min).

The correct band size was checked on a 1.5 % agarose gel (app. 900 bp) and the rest of the PCR-fragment was purified using Biorad spin columns.

The PCR-fragment was cloned into and cloned into pENI1960 cut ScaI using Gateway cloning as recommended by the supplier (Life Technologies) and transformed into E.coli DH10b and sequenced, thus creating an intron-less *Talaromyces emersonii* lipase gene.

Removal of introns from *Thermomyces lbadanensis* lipase gene

4 PCR reactions were run using PWO polymerase and pENI2147 as template (94°C 5 min, 30* (94°C 30 sec., 50°C 30 sec, 72°C 1 min), 72°C 5min) and the following oligoes:

1: 051200j17 and 051200j19 (SEQ ID NO: 27 and 29).

2: 051200j18 and 051200j21 (SEQ ID NO: 28 and 31).

3: 051200j20 and 051200j23 (SEQ ID NO: 30 and 33).

4: 051200j22 and 051200j24 (SEQ ID NO: 32 and 34).

The specific bands were run and purified from a 1.5 % agarose gel. Equal amounts of PCR fragments were mixed along with PWO polymerase, buffer, dNTP, oligo 051200j17 and 051200j24 (SEQ ID NO: 27 and 34, total of 50 µl, as recommended by the supplier) and a second PCR was run (94°C 5 min, 30* (94°C 30 sec., 50°C 30 sec, 72°C 2 min), 72°C 5min).

The correct band size was checked on a 1.5 % agarose gel (app. 900 bp) and the rest of the PCR-fragment was purified using Biorad spin columns

The PCR-fragment was cloned into and cloned into pENI1960 cut ScaI using Gateway cloning as recommended by supplier (life technologies) and transformed into *E.coli* DH10b and sequenced, thus creating intron-less *Thermomyces lbadanensis* lipase gene.

Example 4: Shuffling of lipolytic enzyme genes

Plasmids containing DNA sequences encoding lipolytic enzymes are mixed in equimolar amounts. The following components where mixed in a microtube:

2 µl plasmid mixture (0.15 µg/µl), specific primers flanking the gene (1 pmol/µ), 2 µl 2.5 mM dNTP, 2.5 mM MgCl2, 2 µl 10* taq buffer (Perkin Elmer), 0.5 µl taq enzyme in a total volume of 20 µl.

The tube is set in a Perkin Elmer 2400 thermocycler. The following PCR-program is run:(94°C, 5 minutes) 1 cycle:

(94°C , 30 seconds, 70°C, 0 seconds) 99 cycles(72°C, 2 minutes, 4°C indefinite) 1 cycle

The PCR-reaction is run on a 1.5 % agarose gel. A DNA-band of the specific expected size is cut out of the agarose gel and purified using JETsorb (from GENOMED Inc.). The purified PCR-product is cloned into a TA-vector (from Invitrogen (the original TA cloning kit). The ligated product is transformed into a standard Escherichia coli strain (DH5a).

The shuffled sequences can then be subcloned from the *E. coli* TA vector into the yeast vector pJSOO26 (WO 9928448) as a BamHI-XbaI fragment (see WO 97/07205), and e.g. screened for new shuffled sequences with improved properties, e.g. improved performance in detergents (see WO 97/07205).

Example 5: Shuffling of lipolytic enzyme genes

PCR products of lipolytic enzyme genes are generated as in the previous example and pooled in equimolar amounts. The following mixture is generated in a suitable tube:

1 µl PCR mixture (0.1 µg), decamer random primer (300 pmol), 2 µl 10* Klenow buffer (Promega), 0.25 mM dNTP, 2.5 mM MgCl2 in a total volume of 20 µl.

The mixture is set in a PE2400 thermocycler where the following program is run: 96°C, 5 minutes, 25°C 5 minutes, 0.5 ml Klenow enzyme is added, 25°C 60 minutes, 35°C 90 minutes.

This procedure generates a high number of small DNA polymers originating from all parts of the gene

10 µl is taken out for test on agarose gel.

10 µl PCR mixture (0.25 mM dNTP, 1 µl 10* Taq buffer (Perkin Elmer), 2.5 mM MgCl2, 0.5 µl Taq enzyme) is added to the 10 µl in the tube in the thermocycler. Then the following standard PCR-program is run: (94°C, 5 minutes) 1 cycle, (94°C 30 seconds, 45°C, 30 seconds, 72°C 30 seconds) 25 cycles, 72°C 7 minutes, 4°C indefinite.

The PCR products are run on a 1.5% agarose gel. A clear unbiased smear is seen. DNA between 400 and 800 bp is isolated from the gel.

Half of the purified PCR product is mixed in a tube with two specific primers (40 pmol) flanking the gene of interest, 0.25 mM dNTP, 2 µl 10* Taq buffer, 2.5 mM MgCl2. Then the following standard PCR-program is run: (94°C , 5 minutes) 1 cycle, (94°C 30 seconds, 50°C, 30 seconds, 72°C 30 seconds) 25 cycles, 72°C 7 minutes, 4°C indefinite.

The PCR product is run on a 1.5% agarose gel. A band of the expected size is isolated. Additional PCR is run using specific primers (as mentioned above) in order to amplify the PCR-product before cloning.

The PCR-product and the desired vector are cut with the appropriate restriction enzymes (BamHI/XhoI). The vector and the PCR product are run on a 1.5% agarose gel, and purified from the gel.

The cut PCR-product and the cut vector are mixed in a ligase buffer with T4 DNA ligase (Promega). After overnight ligation at 16°C the mixture is transformed into *E. coli* strain DH5a.

Example 6: Creation of intron-less lipase genes

A number of lipase genes with homology to the *Thermomyces lanuginosus* lipase gene were cloned. These genes were cloned as genomic DNA and were thus known to contain introns.

The intention was to shuffle these genes in order to obtain chimeric genes. In order to obtain the highest possible quality of library, the introns had to be removed. This was done by creating DNA oligo´s matching each flank of an exon as well as having a DNA sequence, which is homologous to the next neighbour exon.

These oligoes were used in standard PCR (as known to a person skilled in the art), thus creating PCR fragments covering each and every exon (coding sequence) in the gene. These PCR fragments were purified from a 1 % agarose gel. The PCR fragments were assembled into a full length gene, in a second PCR using the DNA oligoes flanking the whole gene, as primers.

The PCR fragment containing the full length intron-less gene encoding the lipase was cloned into pENI 1960 as described in pat. appl. PCT/DK02/00050.

The following primers were used to assemble each intron-less gene:

Talaromyces thermophilus: 051200j1, 051200J2, 051200J3, 051200J4, 051200J5, 051200J6, 051200J7 and 051200J8 (SEQ ID NO: 11-18), thus creating pENI2178, when cloned into pENI1960.

Talaromyces emersonii: 051200J9, 051200J10, 051200J11, 051200J12, 051200J13, 051200J14, 051200J15 and 051200J16 (SEQ ID NO: 19-26), thus creating pENI2159, when cloned into pENI1960.

Thermomyces ibadanensis: 051200J17, 051200J18, 051200J19, 051200J20, 051200J21, 051200J22, 051200J23 and 051200J24 (SEQ ID NO: 27-34), thus creating pENI2160, when cloned into pENI1960.

Talaromyces byssochlamydoides: 080201P1, 080201P2, 080201P3, 080201P4, 080201P5, 080201P6, 080201P7 and 080201P8 (SEQ ID NO: 54-61), thus creating pENI2230 when cloned into pENI1960.

Example 7: Shuffling of the intron-less lipase genes

A method using dUTP and uracil-DNA glycosylase was employed in order to make DNA fragments in sufficient quantities for DNA shuffling. The 3 genes *T. lanuginosus*, *T .thermophilus* and *T .ibadanensis* are quite homologous to each other (thus named Group A) as are *T. emersonii* and *T. byssochlamydoides* (named Group B). Thus in order to improve recombination between the two groups the following PCR scheme (see Fig. 1) was employed,

using the following templates: pENI2178, pENI2159, pENI2160, pENI2230, and the *T. lanuginosus* gene cloned into pENI1902 (cut BamHI and SacII) (pat. PCT/DK02/00050).

The following oligonucleotides are shown in Fig. 1: 1298-taka, 19670, 19672, 115120 and 050401P6 (SEQ ID NO: 62-65 and 68). 050401P1 (SEQ ID NO: 66) hybridises to 5` *T. lanuginose* lipase gene. 030501P1 (SEQ ID NO: 67) hybridises to 5´ of the other 4 lipase genes.

The final PCR fragment was cut first with BstEII and then with SfiI, as was the vector pENI2376. pENI2376 is a derivative of pENI1861(pat. PCT/DK02/00050)

The vector and PCR-fragment was purified from a 1 % gel and ligated O/N. The ligated DNA pool was transformed into electro-competent *E.coli* DH10B, thus creating a library of app. 700.000 independent clones.

This library can be screened for activity towards various substrates such as Lecithin, DGDG, triglycerides such as tributyrine, olive oil, PNP-valerate or PNP-palmitate at different conditions such as high pH, low pH, high temperature, in presences of detergent, in the presence of ions or in the absence of ions.

This can be done in order to find, e.g., a thermo-stable lipase, a detergent phospholipase, a detergent lipase with first-wash performance, and no activity at neutral pH and so forth.

DNA- oligoes:

1298-taka:

gcaagcgcgcgcaatacatggtgttttgatcat

19670:

ccccatcctttaactatagcg

19672:

ccacacttctcttccttcctc

115120:

gctttgtgcagggtaaatc

050401P1:

cggccgggccgcggaggccagggatccaccatgaggagctcccttgtgctg

030501P1:

cggccgggccgcggaggccacaagtttgtacaaaaaagcagg

(hybridises to 5´ of the other 4 lipase genes)

050401P6:

cggccgggtcacccccccatcctttaactatagcg

Example 8: Characterization of lipolytic enzymes

Lipolytic enzymes from *Thermomyces ibadanensis* and *Talaromyces thermophilus* were prepared as described above, purified and used for characterization

The specific lipase activity was determined by the LU method described in WO 0032758, and the amount of enzyme protein was determined from the optical density at 280 nm. The specific activity was found to be 3181 LU/mg for the *Th. ibadanensis* lipase and 1000 LU/mg for the *Tal. thermophilus* lipase.

The pH-activity relation was found by determining the lipase by the LU method at pH 5, 6, 7, 8, 9 and 10. Both enzymes were found to have the highest lipase activity at pH 10. The *Th. ibadanensis* lipase showed a broad optimum with more than 50 % of maximum activity in the pH range 6-10 whereas the *Tal. thermophilus* lipase showed a stronger activity drop at lower pH with less than 30 % of maximum activity at pH 5-8.

The thermostability was determined by differential scanning calorimetry (DSC) at pH 5 (50 mM acetate buffer), pH 7 (50 mM HEPES buffer) and pH 10 (50 mM glycine buffer) with a scan rate of 90°C/hr. The temperature at the top of the denaturation peak (T_d) was found to be as follows:

pH	T_d (°C)	
	T. ibadanesis	*T. thermophilus*
5	74*	72*
7	72	75
10	64	69

Example 9: Lysophospholipase activity

Purified lipolytic enzymes from *T. ibadanensis* and *T. thermos* were tested by incubating with lysolecithin as substrate at pH 5 and 7, and the extent of reaction was followed by use of NEFA kit.

The results were that the enzyme from *T. ibadanensis* showed high lysophospholipase activity at pH 5 and some activity at pH 7. The enzyme from *T. thermos* showed a slight activity.

3	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
3-1	page	4
3-2	line	11-16
3-3	Identification of Deposit	
3-3-1	Name of depositary institution	DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
3-3-2	Address of depositary institution	Mascheroder Weg 1b, D-38124 Braunschweig, Germany
3-3-3	Date of deposit	08 February 2001 (08.02.2001)
3-3-4	Accession Number	DSMZ 14049
3-4	Additional Indications	NONE
3-5	Designated States for Which Indications are Made	all designated States
3-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE
4	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
4-1	page	4
4-2	line	11-16
4-3	Identification of Deposit	
4-3-1	Name of depositary institution	DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
4-3-2	Address of depositary institution	Mascheroder Weg 1b, D-38124 Braunschweig, Germany
4-3-3	Date of deposit	08 February 2001 (08.02.2001)
4-3-4	Accession Number	DSMZ 14051
4-4	Additional Indications	NONE
4-5	Designated States for Which Indications are Made	all designated States
4-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE

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0-1	Form - PCT/RO/134 (EASY) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)	
0-1-1	Prepared using	PCT-EASY Version 2.92 (updated 01.01.2002)
0-2	International Application No.	
0-3	Applicant's or agent's file reference	10130-WO

1	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
1-1	page	4
1-2	line	11-16
1-3	Identification of Deposit	
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1-3-2	Address of depositary institution	Mascheroder Weg 1b, D-38124 Braunschweig, Germany
1-3-3	Date of deposit	08 February 2001 (08.02.2001)
1-3-4	Accession Number	DSMZ 14047
1-4	Additional Indications	NONE
1-5	Designated States for Which Indications are Made	all designated States
1-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE
2	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
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2-2	line	11-16
2-3	Identification of Deposit	
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2-3-2	Address of depositary institution	Mascheroder Weg 1b, D-38124 Braunschweig, Germany
2-3-3	Date of deposit	08 February 2001 (08.02.2001)
2-3-4	Accession Number	DSMZ 14048
2-4	Additional Indications	NONE
2-5	Designated States for Which Indications are Made	all designated States
2-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE

0-5-1	Authorized officer	

CLAIMS

1. A method of producing a lipolytic enzyme which comprises:

a) shuffling at least two polynucleotides which comprise:

i) a polynucleotide encoding a polypeptide which has lipolytic enzyme activity and has an amino acid sequence having at least 90 % identity with the mature peptide of SEQ ID NO: 2, and

ii) a polynucleotide encoding a polypeptide which has lipolytic enzyme activity and has an amino acid sequence having 55-90 % identity with the mature peptide of SEQ ID NO: 2

b) expressing the shuffled polynucleotides to form recombinant polypeptides,

c) screening the polypeptides to select a polypeptide having lipolytic enzyme activity, and

d) producing the selected polypeptide.

2. The method of claim 1 wherein the amino acid sequence encoded by polynucleotide (ii) has at least 90 % identity to the mature part of SEQ ID NO: 4, 6, 8 or10.

3. The method of claim 1 or 2 wherein the polynucleotides comprise a polynucleotide having a nucleotide sequence having at least 90 % identity to the mature part of SEQ ID NO: 1, 3, 5, 7 or 9.

4. A polynucleotide which comprises a nucleotide sequence which encodes a lipolytic enzyme and which:

a) is a DNA sequence cloned into a plasmid present in *Escherichia coli* deposit number DSM 14047, 14048, 14049, or 14051, or

b) is the DNA sequence encoding a mature peptide shown in SEQ ID NO: 3, 5, 7 or 9 or can be derived therefrom by substitution, deletion, and/or insertion of one or more nucleotides, or

c) has at least 90 % identity with the DNA sequence encoding a mature peptide shown in SEQ ID NO: 3, at least 80 % identity with the DNA sequence encoding a mature peptide shown in SEQ ID NO: 5, at least 65 % identity with the DNA sequence encoding a mature peptide shown in SEQ ID NO: 7 or at least 60 % identity with the DNA sequence encoding a mature peptide shown in SEQ ID NO: 9, or

d) is an allelic variant of the DNA sequence encoding a mature peptide shown in SEQ ID NO: 3, 5, 7 or 9; or

e) hybridizes under high stringency conditions with a complementary strand of the nucleic acid sequence encoding a mature peptide shown in SEQ ID NO: 3, 5, 7 or 9, or a subsequence thereof having at least 100 nucleotides.

5. The polynucleotide of claim 4 which further comprises one or more control sequences which are operably linked to said nucleotide sequence and capable of directing the expression of the lipolytic enzyme in a suitable expression host.

6. A recombinant expression vector comprising the polynucleotide of claim 5, a promoter, and transcriptional and translational stop signals.

7. A recombinant host cell transformed with the polynucleotide of claim 5 or the vector of claim 6.

8. A method for producing a polypeptide having lipolytic enzyme activity comprising cultivating the host cell of claim 7 under conditions conducive to production of the polypeptide, and recovering the polypeptide.

9. A polypeptide which has lipolytic enzyme activity and which:

f) is encoded by a DNA sequence cloned into a plasmid present in *Escherichia coli* deposit number DSM 14047 or 14049, or

g) has an amino acid sequence which is the mature peptide of SEQ ID NO: 6 or10, or can be derived therefrom by substitution, deletion, and/or insertion of one or more amino acids, or

h) has an amino acid sequence which has at least 80 % identity with the mature peptide of SEQ ID NO: 6 or at least 60 % identity with the mature peptide of SEQ ID NO: 10, or

i) is immunologically reactive with an antibody raised against the mature peptide of SEQ ID NO: 6 or 10 in purified form, or

j) is an allelic variant of the mature peptide of SEQ ID NO: 6 or10; or

k) is encoded by a nucleic acid sequence which hybridizes under high stringency conditions with a complementary strand of the nucleic acid sequence encoding a mature peptide shown in SEQ ID NO: 5 or 9, or a subsequence thereof having at least 100 nucleotides.

10. The polypeptide of claim 9 which is native to a strain of *Talaromyces* or *Thermomyces*, particularly *Thermomyces lbadanensis* or *Talaromyces byssochlamydoides*.

11. A nucleic acid sequence comprising a nucleic acid sequence which encodes the polypeptide of claim 9 or 10.

12. A process for hydrolyzing the fatty acyl group in a lysophospholipid, comprising treating the lysophospholipid with a polypeptide which has lysophospholipase activity and which:

a) is encoded by a DNA sequence cloned into a plasmid present in *Escherichia coli* deposit number DSM 14047, 14048, 14049 or 14051, or

b) has an amino acid sequence which is the mature peptide of SEQ ID NO: 4, 6, 8 or10, or can be derived therefrom by substitution, deletion, and/or insertion of one or more amino acids, or

c) has an amino acid sequence which has at least 55 % identity with the mature peptide of SEQ ID NO: 4, 6, 8 or 10, or

d) is immunologically reactive with an antibody raised against the mature peptide of SEQ ID NO: 4, 6, 8 or 10 in purified form, or

e) is an allelic variant of the mature peptide of SEQ ID NO: 4, 6, 8 or 10; or

f) is encoded by a nucleic acid sequence which hybridizes under high stringency conditions with a complementary strand of the nucleic acid sequence encoding a mature peptide shown in SEQ ID NO: 3, 5, 7 or 9, or a subsequence thereof having at least 100 nucleotides.

13. A process according to claim 12 for improving the filterability of an aqueous solution or slurry of carbohydrate origin which contains lysophospholipid.

14. The process of the preceding claim wherein the solution or slurry contains a starch hydrolysate, particularly a wheat starch hydrolysate.

15. A detergent composition comprising a surfactant and a polypeptide which has lipolytic enzyme activity and which:

a) is encoded by a DNA sequence cloned into a plasmid present in *Escherichia coli* deposit number DSM 14047, 14048, 14049 or 14051, or

b) has an amino acid sequence which is the mature peptide of SEQ ID NO: 4, 6, 8 or 10, or can be derived therefrom by substitution, deletion, and/or insertion of one or more amino acids, or

c) has an amino acid sequence which has at least 55 % identity with the mature peptide of SEQ ID NO: 4, 6, 8 or 10, or

d) is immunologically reactive with an antibody raised against the mature peptide of SEQ ID NO: 4, 6, 8 or 10 in purified form, or

e) is an allelic variant of the mature peptide of SEQ ID NO: 4, 6, 8 or10; or

f) is encoded by a nucleic acid sequence which hybridizes under high stringency conditions with a complementary strand of the nucleic acid sequence encoding a mature peptide shown in SEQ ID NO: 3, 5, 7 or 9, or a subsequence thereof having at least 100 nucleotides.

16. A flour composition comprising flour and a polypeptide which has lipolytic enzyme activity and which:

a) is encoded by a DNA sequence cloned into a plasmid present in *Escherichia coli* deposit number DSM 14047, 14048, 14049 or 14051, or

b) has an amino acid sequence which is the mature peptide of SEQ ID NO: 4, 6, 8 or 10, or can be derived therefrom by substitution, deletion, and/or insertion of one or more amino acids, or

c) has an amino acid sequence which has at least 55 % identity with the mature peptide of SEQ ID NO: 4, 6, 8 or 10, or

d) is immunologically reactive with an antibody raised against the mature peptide of SEQ ID NO: 4, 6, 8 or 10 in purified form, or

e) is an allelic variant of the mature peptide of SEQ ID NO: 4, 6, 8 or 10; or

f) is encoded by a nucleic acid sequence which hybridizes under high stringency conditions with a complementary strand of the nucleic acid sequence encoding a mature peptide shown in SEQ ID NO: 3, 5, 7 or 9, or a subsequence thereof having at least 100 nucleotides.

17. A process for producing a dough or a baked product made from dough, comprising adding to the dough a polypeptide which has lipolytic enzyme activity and which:

g) is encoded by a DNA sequence cloned into a plasmid present in *Escherichia coli* deposit number DSM 14047, 14048, 14049 or 14051, or

h) has an amino acid sequence which is the mature peptide of SEQ ID NO: 4, 6, 8 or 10, or can be derived therefrom by substitution, deletion, and/or insertion of one or more amino acids, or

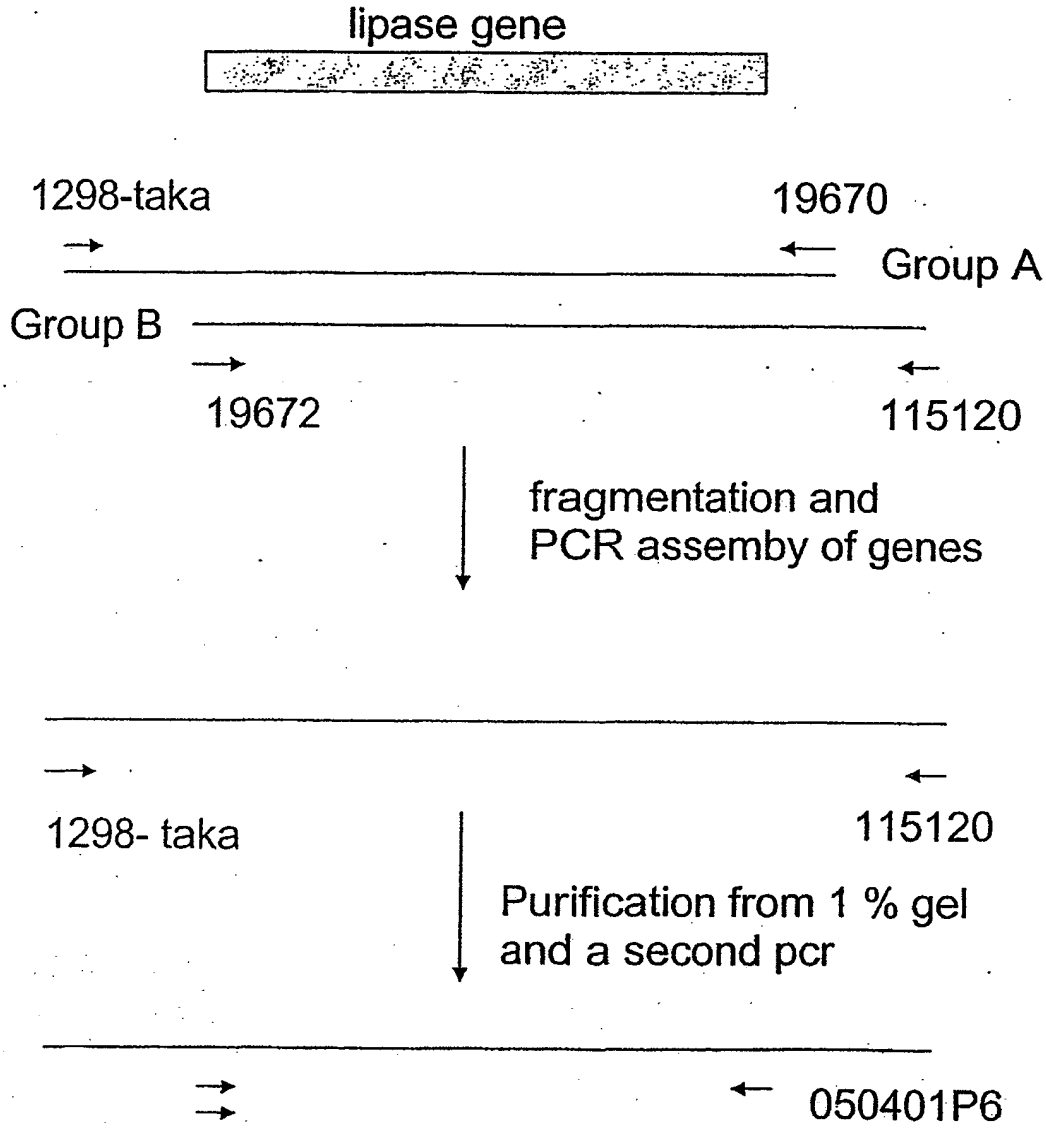
i) has an amino acid sequence which has at least 55 % identity with the mature peptide of SEQ ID NO: 4, 6, 8 or 10, or

j) is immunologically reactive with an antibody raised against the mature peptide of SEQ ID NO: 4, 6, 8 or 10 in purified form, or

k) is an allelic variant of the mature peptide of SEQ ID NO: 4, 6, 8 or 10; or

l) is encoded by a nucleic acid sequence which hybridizes under high stringency conditions with a complementary strand of the nucleic acid sequence encoding a mature peptide shown in SEQ ID NO: 3, 5, 7 or 9, or a subsequence thereof having at least 100 nucleotides.

1/1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Group A
Group B
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Purification from 1 % gel
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FIG. 1

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-20 -15 -10

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gcc agt cct att cgt cga gag gtc tcg cag gat ctg ttt aac cag ttc        96
Ala Ser Pro Ile Arg Arg Glu Val Ser Gln Asp Leu Phe Asn Gln Phe
 -5                  -1  1                   5                  10

aat ctc ttt gca cag tat tct gca gcc gca tac tgc gga aaa aac aat       144
Asn Leu Phe Ala Gln Tyr Ser Ala Ala Ala Tyr Cys Gly Lys Asn Asn
             15                  20                  25

gat gcc cca gct ggt aca aac att acg tgc acg gga aat gcc tgc ccc       192
Asp Ala Pro Ala Gly Thr Asn Ile Thr Cys Thr Gly Asn Ala Cys Pro
         30                  35                  40

gag gta gag aag gcg gat gca acg ttt ctc tac tcg ttt gaa gac tct       240
Glu Val Glu Lys Ala Asp Ala Thr Phe Leu Tyr Ser Phe Glu Asp Ser
     45                  50                  55

gga gtg ggc gat gtc acc ggc ttc ctt gct ctc gac aac acg aac aaa       288
Gly Val Gly Asp Val Thr Gly Phe Leu Ala Leu Asp Asn Thr Asn Lys
 60                  65                  70

ttg atc gtc ctc tct ttc cgt ggc tct cgt tcc ata gag aac tgg atc       336
Leu Ile Val Leu Ser Phe Arg Gly Ser Arg Ser Ile Glu Asn Trp Ile
75                  80                  85                  90

ggg aat ctt aac ttc gac ttg aaa gaa ata aat gac att tgc tcc ggc       384
Gly Asn Leu Asn Phe Asp Leu Lys Glu Ile Asn Asp Ile Cys Ser Gly
             95                 100                 105

tgc agg gga cat gac ggc ttc act tcg tcc tgg agg tct gta gcc gat       432
Cys Arg Gly His Asp Gly Phe Thr Ser Ser Trp Arg Ser Val Ala Asp
        110                 115                 120

acg tta agg cag aag gtg gag gat gct gtg agg gag cat ccc gac tat       480
Thr Leu Arg Gln Lys Val Glu Asp Ala Val Arg Glu His Pro Asp Tyr
    125                 130                 135

cgc gtg gtg ttt acc gga cat agc ttg ggt ggt gca ttg gca act gtt       528
Arg Val Val Phe Thr Gly His Ser Leu Gly Gly Ala Leu Ala Thr Val
140                 145                 150

gcc gga gca gac ctg cgt gga aat ggg tat gat atc gac gtg ttt tca       576
Ala Gly Ala Asp Leu Arg Gly Asn Gly Tyr Asp Ile Asp Val Phe Ser
155                 160                 165                 170

tat ggc gcc ccc cga gtc gga aac agg gct ttt gca gaa ttc ctg acc       624
Tyr Gly Ala Pro Arg Val Gly Asn Arg Ala Phe Ala Glu Phe Leu Thr
             175                 180                 185

gta cag acc ggc gga aca ctc tac cgc att acc cac acc aat gat att       672
Val Gln Thr Gly Gly Thr Leu Tyr Arg Ile Thr His Thr Asn Asp Ile
        190                 195                 200

gtc cct aga ctc ccg ccg cgc gaa ttc ggt tac agc cat tct agc cca       720
Val Pro Arg Leu Pro Pro Arg Glu Phe Gly Tyr Ser His Ser Ser Pro
    205                 210                 215

gag tac tgg atc aaa tct gga acc ctt gtc ccc gtc acc cga aac gat       768
Glu Tyr Trp Ile Lys Ser Gly Thr Leu Val Pro Val Thr Arg Asn Asp
220                 225                 230

atc gtg aag ata gaa ggc atc gat gcc acc ggc ggc aat aac cag cct       816
Ile Val Lys Ile Glu Gly Ile Asp Ala Thr Gly Gly Asn Asn Gln Pro
235                 240                 245                 250

aac att ccg gat atc cct gcg cac cta tgg tac ttc ggg tta att ggg       864
Asn Ile Pro Asp Ile Pro Ala His Leu Trp Tyr Phe Gly Leu Ile Gly
             255                 260                 265
```

aca tgt ctt tagtggccgg cgcggctggg tccgactcta gcgagctcga gatct 918
Thr Cys Leu

<210> 2

<211> 291

<212> PRT

<213> Thermomyces lanuginosus

<400> 2

Met Arg Ser Ser Leu Val Leu Phe Phe Val Ser Ala Trp Thr Ala Leu
-20 -15 -10

Ala Ser Pro Ile Arg Arg Glu Val Ser Gln Asp Leu Phe Asn Gln Phe
-5 -1 1 5 10

Asn Leu Phe Ala Gln Tyr Ser Ala Ala Ala Tyr Cys Gly Lys Asn Asn
15 20 25

Asp Ala Pro Ala Gly Thr Asn Ile Thr Cys Thr Gly Asn Ala Cys Pro
30 35 40

Glu Val Glu Lys Ala Asp Ala Thr Phe Leu Tyr Ser Phe Glu Asp Ser
45 50 55

Gly Val Gly Asp Val Thr Gly Phe Leu Ala Leu Asp Asn Thr Asn Lys
60 65 70

Leu Ile Val Leu Ser Phe Arg Gly Ser Arg Ser Ile Glu Asn Trp Ile
75 80 85 90

Gly Asn Leu Asn Phe Asp Leu Lys Glu Ile Asn Asp Ile Cys Ser Gly
95 100 105

Cys Arg Gly His Asp Gly Phe Thr Ser Ser Trp Arg Ser Val Ala Asp
110 115 120

Thr Leu Arg Gln Lys Val Glu Asp Ala Val Arg Glu His Pro Asp Tyr
125 130 135

Arg Val Val Phe Thr Gly His Ser Leu Gly Gly Ala Leu Ala Thr Val
140 145 150

Ala Gly Ala Asp Leu Arg Gly Asn Gly Tyr Asp Ile Asp Val Phe Ser
155 160 165 170

Tyr Gly Ala Pro Arg Val Gly Asn Arg Ala Phe Ala Glu Phe Leu Thr
175 180 185

Val Gln Thr Gly Gly Thr Leu Tyr Arg Ile Thr His Thr Asn Asp Ile
190 195 200

Val Pro Arg Leu Pro Pro Arg Glu Phe Gly Tyr Ser His Ser Ser Pro
205 210 215

Glu Tyr Trp Ile Lys Ser Gly Thr Leu Val Pro Val Thr Arg Asn Asp
220 225 230

Ile Val Lys Ile Glu Gly Ile Asp Ala Thr Gly Gly Asn Asn Gln Pro
235 240 245 250

Asn Ile Pro Asp Ile Pro Ala His Leu Trp Tyr Phe Gly Leu Ile Gly
255 260 265

Thr Cys Leu

<210> 3

<211> 1083

<212> DNA

<213> Talaromyces thermophilus

<220>

<221> CDS

<222> (1)..(67)

<223>

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<222> (139)..(307)

<223>

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<222> (778)..(1080)

<223>

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<221> mat_peptide

<222> (67)..()

<223>

<400> 3

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atg agg agc tcg ctc gtg ctg ttc ttc gtt tct gcg tgg acg gcc ttg       48
Met Arg Ser Ser Leu Val Leu Phe Phe Val Ser Ala Trp Thr Ala Leu
        -20                 -15                 -10

gcc agt cct gtc cga cga g gtatgtaaat cacggggtat acttttcatg           97
Ala Ser Pro Val Arg Arg
    -5              -1

cattgcatgt cgaacctgct gtactaagat tgcgcgcaca g ag  gtc tcg cag gat    152
                                              Glu Val Ser Gln Asp
                                                                5

ctg ttt gac cag ttc aac ctc ttt gcg cag tac tcg gcg gcc gca tac      200
Leu Phe Asp Gln Phe Asn Leu Phe Ala Gln Tyr Ser Ala Ala Ala Tyr
                10                  15                  20

tgc gcg aag aac aac gat gcc ccg gca ggt ggg aac gta acg tgc agg      248
Cys Ala Lys Asn Asn Asp Ala Pro Ala Gly Gly Asn Val Thr Cys Arg
            25                  30                  35

gga agt att tgc ccc gag gta gag aag gcg gat gca acg ttt ctc tac      296
Gly Ser Ile Cys Pro Glu Val Glu Lys Ala Asp Ala Thr Phe Leu Tyr
        40                  45                  50

tcg ttt gag ga  gtaggtgtca acaagagtac aggcacccgt agtagaaata          347
Ser Phe Glu Asp
    55

gcagactaac tgggaaatgt ag t tct gga gtt ggc gat gtc acc ggg ttc      397
                          Ser Gly Val Gly Asp Val Thr Gly Phe
                                  60                  65

ctt gct ctc gac aac acg aac aga ctg atc gtc ctc tct ttc cgc ggc      445
Leu Ala Leu Asp Asn Thr Asn Arg Leu Ile Val Leu Ser Phe Arg Gly
            70                  75                  80

tct cgt tcc ctg gaa aac tgg atc ggg aat atc aac ttg gac ttg aaa      493
Ser Arg Ser Leu Glu Asn Trp Ile Gly Asn Ile Asn Leu Asp Leu Lys
        85                  90                  95

gga att gac gac atc tgc tct ggc tgc aag gga cat gac ggc ttc act      541
Gly Ile Asp Asp Ile Cys Ser Gly Cys Lys Gly His Asp Gly Phe Thr
    100                 105                 110

tcc tcc tgg agg tcc gtt gcc aat acc ttg act cag caa gtg cag aat      589
Ser Ser Trp Arg Ser Val Ala Asn Thr Leu Thr Gln Gln Val Gln Asn
115                 120                 125                 130

gct gtg agg gag cat ccc gac tac cgc gtc gtc ttc act ggg cac agc      637
                                    5
```

```
Ala Val Arg Glu His Pro Asp Tyr Arg Val Val Phe Thr Gly His Ser
                135                 140                 145

ttg ggt ggt gca ttg gca act gtg gcc ggg gca tct ctg cgt gga aat      685
Leu Gly Gly Ala Leu Ala Thr Val Ala Gly Ala Ser Leu Arg Gly Asn
            150                 155                 160

ggg tac gat ata gat gtg gtatgtagga aaaatgatcc ccgtggagcg            733
Gly Tyr Asp Ile Asp Val
        165

gtcatgtgga aatgtgcagg ggtgtctaat acacagacca acag ttc tca tat ggc     789
                                              Phe Ser Tyr Gly
                                                  170

gct ccc cgc gtc gga aac agg gct ttt gcg gaa ttc ctg acc gca cag      837
Ala Pro Arg Val Gly Asn Arg Ala Phe Ala Glu Phe Leu Thr Ala Gln
        175                 180                 185

acc ggc ggc acc ttg tac cgc atc acc cac acc aat gat att gtc ccc      885
Thr Gly Gly Thr Leu Tyr Arg Ile Thr His Thr Asn Asp Ile Val Pro
    190                 195                 200

aga ctc ccg cca cgc gaa ttg ggt tac agc cat tct agc cca gag tat      933
Arg Leu Pro Pro Arg Glu Leu Gly Tyr Ser His Ser Ser Pro Glu Tyr
205                 210                 215                 220

tgg atc acg tct gga acc ctc gtc cca gtg acc aag aac gat atc gtc      981
Trp Ile Thr Ser Gly Thr Leu Val Pro Val Thr Lys Asn Asp Ile Val
                225                 230                 235

aag gtg gag ggc atc gat tcc acc gat gga aac aac cag cca aat acc     1029
Lys Val Glu Gly Ile Asp Ser Thr Asp Gly Asn Asn Gln Pro Asn Thr
            240                 245                 250

ccg gac att gct gcg cac cta tgg tac ttc ggg tca atg gcg acg tgt     1077
Pro Asp Ile Ala Ala His Leu Trp Tyr Phe Gly Ser Met Ala Thr Cys
        255                 260                 265

ttg taa                                                            1083
Leu
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<210> 4

<211> 291

<212> PRT

<213> Talaromyces thermophilus

<400> 4

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Met Arg Ser Ser Leu Val Leu Phe Phe Val Ser Ala Trp Thr Ala Leu
        -20                 -15                 -10

Ala Ser Pro Val Arg Arg Glu Val Ser Gln Asp Leu Phe Asp Gln Phe
    -5              -1  1                   5                  10

Asn Leu Phe Ala Gln Tyr Ser Ala Ala Ala Tyr Cys Ala Lys Asn Asn
                15                  20                  25
```

Asp Ala Pro Ala Gly Gly Asn Val Thr Cys Arg Gly Ser Ile Cys Pro
30 35 40

Glu Val Glu Lys Ala Asp Ala Thr Phe Leu Tyr Ser Phe Glu Asp Ser
45 50 55

Gly Val Gly Asp Val Thr Gly Phe Leu Ala Leu Asp Asn Thr Asn Arg
60 65 70

Leu Ile Val Leu Ser Phe Arg Gly Ser Arg Ser Leu Glu Asn Trp Ile
75 80 85 90

Gly Asn Ile Asn Leu Asp Leu Lys Gly Ile Asp Asp Ile Cys Ser Gly
95 100 105

Cys Lys Gly His Asp Gly Phe Thr Ser Ser Trp Arg Ser Val Ala Asn
110 115 120

Thr Leu Thr Gln Gln Val Gln Asn Ala Val Arg Glu His Pro Asp Tyr
125 130 135

Arg Val Val Phe Thr Gly His Ser Leu Gly Gly Ala Leu Ala Thr Val
140 145 150

Ala Gly Ala Ser Leu Arg Gly Asn Gly Tyr Asp Ile Asp Val Phe Ser
155 160 165 170

Tyr Gly Ala Pro Arg Val Gly Asn Arg Ala Phe Ala Glu Phe Leu Thr
175 180 185

Ala Gln Thr Gly Gly Thr Leu Tyr Arg Ile Thr His Thr Asn Asp Ile
190 195 200

Val Pro Arg Leu Pro Pro Arg Glu Leu Gly Tyr Ser His Ser Ser Pro
205 210 215

Glu Tyr Trp Ile Thr Ser Gly Thr Leu Val Pro Val Thr Lys Asn Asp
220 225 230

Ile Val Lys Val Glu Gly Ile Asp Ser Thr Asp Gly Asn Asn Gln Pro
235 240 245 250

Asn Thr Pro Asp Ile Ala Ala His Leu Trp Tyr Phe Gly Ser Met Ala
255 260 265

Thr Cys Leu

<210> 5

<211> 1070

<212> DNA

<213> Thermomyces ibadanensis

<220>

<221> CDS

<222> (1)..(67)

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<221> CDS

<222> (128)..(296)

<223>

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<222> (357)..(690)

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<221> CDS

<222> (765)..(1067)

<223>

<220>

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<222> (67)..()

<223>

<400> 5

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atg cgg agc tcc ctc gtg ctg ttc ttc ctc tct gcg tgg acg gcc ttg       48
Met Arg Ser Ser Leu Val Leu Phe Phe Leu Ser Ala Trp Thr Ala Leu
       -20                 -15                 -10

gcg cgg cct gtt cga cga g gtatgtagca agggacacta ttacatgttg           97
Ala Arg Pro Val Arg Arg
    -5              -1

accttggtga ttctaagact gcatgcgcag cg  gtt ccg caa gat ctg ctc gac     150
                               Ala Val Pro Gln Asp Leu Leu Asp
                                           5
```

```
cag ttt gaa ctc ttt tca caa tat tcg gcg gcc gca tac tgt gcg gca      198
Gln Phe Glu Leu Phe Ser Gln Tyr Ser Ala Ala Ala Tyr Cys Ala Ala
    10                  15                  20

aac aat cat gct cca gtg ggc tca gac gta acg tgc tcg gag aat gtc      246
Asn Asn His Ala Pro Val Gly Ser Asp Val Thr Cys Ser Glu Asn Val
25                  30                  35                  40

tgc cct gag gta gat gcg gcg gac gca acg ttt ctc tat tct ttt gaa      294
Cys Pro Glu Val Asp Ala Ala Asp Ala Thr Phe Leu Tyr Ser Phe Glu
                45                  50                  55

ga  gtgggtgtcg acaaagcaca gagacagtag tagagacagc agtctaactg          346
Asp

agatgtgcag t tct gga tta ggc gat gtt acc ggc ctt ctc gct ctc gac     396
             Ser Gly Leu Gly Asp Val Thr Gly Leu Leu Ala Leu Asp
                    60                  65                  70

aac acg aat aaa ctg atc gtc ctc tct ttc cgc ggc tct cgc tca gta      444
Asn Thr Asn Lys Leu Ile Val Leu Ser Phe Arg Gly Ser Arg Ser Val
                75                  80                  85

gag aac tgg atc gcg aac ctc gcc gcc gac ctg aca gaa ata tct gac      492
Glu Asn Trp Ile Ala Asn Leu Ala Ala Asp Leu Thr Glu Ile Ser Asp
            90                  95                  100

atc tgc tcc ggc tgc gag ggg cat gtc ggc ttc gtt act tct tgg agg      540
Ile Cys Ser Gly Cys Glu Gly His Val Gly Phe Val Thr Ser Trp Arg
        105                 110                 115

tct gta gcc gac act ata agg gag cag gtg cag aat gcc gtg aac gag      588
Ser Val Ala Asp Thr Ile Arg Glu Gln Val Gln Asn Ala Val Asn Glu
    120                 125                 130

cat ccc gat tac cgc gtg gtc ttt acc gga cat agc ttg gga ggc gca      636
His Pro Asp Tyr Arg Val Val Phe Thr Gly His Ser Leu Gly Gly Ala
135                 140                 145                 150

ctg gca act att gcc gca gca gct ctg cga gga aat gga tac aat atc      684
Leu Ala Thr Ile Ala Ala Ala Ala Leu Arg Gly Asn Gly Tyr Asn Ile
                155                 160                 165

gac gtg gtatgtggga agaagccacc cagacaaaca attatgtgga aacatgcaag      740
Asp Val

gatggctaat acacggtcca acag ttc tca tat ggc gcg ccc cgc gtc ggt      791
                          Phe Ser Tyr Gly Ala Pro Arg Val Gly
                             170                 175

aac agg gca ttt gca gaa ttc ctg acc gca cag acg ggc ggc acc ctg      839
Asn Arg Ala Phe Ala Glu Phe Leu Thr Ala Gln Thr Gly Gly Thr Leu
        180                 185                 190

tat cgc atc acc cat acc aat gat atc gtc cct aga ctc cct cct cga      887
Tyr Arg Ile Thr His Thr Asn Asp Ile Val Pro Arg Leu Pro Pro Arg
    195                 200                 205

gac tgg ggt tac agc cac tct agc ccg gag tac tgg gtc acg tct ggt      935
Asp Trp Gly Tyr Ser His Ser Ser Pro Glu Tyr Trp Val Thr Ser Gly
210                 215                 220                 225

aac gac gtc cca gtg acc gca aac gac atc acc gtc gtg gag ggc atc      983
Asn Asp Val Pro Val Thr Ala Asn Asp Ile Thr Val Val Glu Gly Ile
                230                 235                 240
```

```
gat tcc acc gac ggg aac aac cag ggg aat atc cca gac atc cct tcg      1031
Asp Ser Thr Asp Gly Asn Asn Gln Gly Asn Ile Pro Asp Ile Pro Ser
            245                 250                 255

cat cta tgg tat ttc ggt ccc att tca gag tgt gat tag                1070
His Leu Trp Tyr Phe Gly Pro Ile Ser Glu Cys Asp
        260                 265
```

<210> 6

<211> 291

<212> PRT

<213> Thermomyces ibadanensis

<400> 6

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Met Arg Ser Ser Leu Val Leu Phe Phe Leu Ser Ala Trp Thr Ala Leu
        -20                 -15                 -10

Ala Arg Pro Val Arg Arg Ala Val Pro Gln Asp Leu Leu Asp Gln Phe
    -5              -1  1               5                   10

Glu Leu Phe Ser Gln Tyr Ser Ala Ala Ala Tyr Cys Ala Ala Asn Asn
                15                  20                  25

His Ala Pro Val Gly Ser Asp Val Thr Cys Ser Glu Asn Val Cys Pro
            30                  35                  40

Glu Val Asp Ala Ala Asp Ala Thr Phe Leu Tyr Ser Phe Glu Asp Ser
        45                  50                  55

Gly Leu Gly Asp Val Thr Gly Leu Leu Ala Leu Asp Asn Thr Asn Lys
    60                  65                  70

Leu Ile Val Leu Ser Phe Arg Gly Ser Arg Ser Val Glu Asn Trp Ile
75                  80                  85                  90

Ala Asn Leu Ala Ala Asp Leu Thr Glu Ile Ser Asp Ile Cys Ser Gly
                95                  100                 105

Cys Glu Gly His Val Gly Phe Val Thr Ser Trp Arg Ser Val Ala Asp
            110                 115                 120

Thr Ile Arg Glu Gln Val Gln Asn Ala Val Asn Glu His Pro Asp Tyr
        125                 130                 135

Arg Val Val Phe Thr Gly His Ser Leu Gly Gly Ala Leu Ala Thr Ile
    140                 145                 150

Ala Ala Ala Ala Leu Arg Gly Asn Gly Tyr Asn Ile Asp Val Phe Ser
155                 160                 165                 170
```

Tyr Gly Ala Pro Arg Val Gly Asn Arg Ala Phe Ala Glu Phe Leu Thr
175 180 185

Ala Gln Thr Gly Gly Thr Leu Tyr Arg Ile Thr His Thr Asn Asp Ile
190 195 200

Val Pro Arg Leu Pro Pro Arg Asp Trp Gly Tyr Ser His Ser Ser Pro
205 210 215

Glu Tyr Trp Val Thr Ser Gly Asn Asp Val Pro Val Thr Ala Asn Asp
220 225 230

Ile Thr Val Val Glu Gly Ile Asp Ser Thr Asp Gly Asn Asn Gln Gly
235 240 245 250

Asn Ile Pro Asp Ile Pro Ser His Leu Trp Tyr Phe Gly Pro Ile Ser
255 260 265

Glu Cys Asp

<210> 7
<211> 1064
<212> DNA
<213> Talaromyces emersonii

<220>
<221> CDS
<222> (1)..(88)
<223>

<220>
<221> mat_peptide
<222> (88)..()
<223>

<220>
<221> CDS
<222> (142)..(310)
<223>

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<221> CDS

<222> (362)..(695)

<223>

<220>

<221> CDS

<222> (756)..(1061)

<223>

<400> 7

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atg ttc aaa tcg gcc gct gtg cgg gcc att gct gcc ctc gga ctg act      48
Met Phe Lys Ser Ala Ala Val Arg Ala Ile Ala Ala Leu Gly Leu Thr
                -25                 -20                 -15

gcg tca gtc ttg gct gct cct gtt gaa ctg ggc cgt cga g gtaaggaagc      98
Ala Ser Val Leu Ala Ala Pro Val Glu Leu Gly Arg Arg
            -10                 -5                  -1

atgacggaga gaacaccctg tgcgacctgc tgacatcctt cag at  gtt tct cag      152
                                                Asp Val Ser Gln

gac ctc ttc gac cag ctc aat ctt ttc gag cag tac tcg gcg gct gcg      200
Asp Leu Phe Asp Gln Leu Asn Leu Phe Glu Gln Tyr Ser Ala Ala Ala
5                   10                  15                  20

tac tgt tca gct aac aat gag gcc tct gcc ggc acg gca atc tct tgc      248
Tyr Cys Ser Ala Asn Asn Glu Ala Ser Ala Gly Thr Ala Ile Ser Cys
                25                  30                  35

tcc gca ggc aat tgc ccg ttg gtc cag cag gct gga gca acc atc ctg      296
Ser Ala Gly Asn Cys Pro Leu Val Gln Gln Ala Gly Ala Thr Ile Leu
            40                  45                  50

tat tca ttc aac aa  gtgggtgtca cggaaaagat tgttgatacc aacatgttga      350
Tyr Ser Phe Asn Asn
        55

cgtgttgtca g c att ggc tct ggc gat gtg acg ggt ttt ctc gct ctc      398
              Ile Gly Ser Gly Asp Val Thr Gly Phe Leu Ala Leu
                    60                  65

gac tcg acg aat caa ttg atc gtc ttg tca ttc cgg gga tca gag act      446
Asp Ser Thr Asn Gln Leu Ile Val Leu Ser Phe Arg Gly Ser Glu Thr
70                  75                  80                  85

ctc gaa aac tgg atc gct gac ctg gaa gct gac ctg gtc gat gcc tct      494
Leu Glu Asn Trp Ile Ala Asp Leu Glu Ala Asp Leu Val Asp Ala Ser
                90                  95                  100

gcc atc tgt tcc ggc tgt gaa gca cac gat ggg ttc ctt tca tcc tgg      542
Ala Ile Cys Ser Gly Cys Glu Ala His Asp Gly Phe Leu Ser Ser Trp
            105                 110                 115

aat tca gtc gcc agc act ctg aca tcc aaa atc tcg tcg gcc gtc aac      590
```

```
Asn Ser Val Ala Ser Thr Leu Thr Ser Lys Ile Ser Ser Ala Val Asn
        120                 125                 130

gaa cat ccc agc tac aag ctg gtc ttc acc ggc cac agt ctc gga gcc      638
Glu His Pro Ser Tyr Lys Leu Val Phe Thr Gly His Ser Leu Gly Ala
    135                 140                 145

gcc ttg gct aca ctt gga gcc gtt tct ctt aga gag agc gga tat aat      686
Ala Leu Ala Thr Leu Gly Ala Val Ser Leu Arg Glu Ser Gly Tyr Asn
150                 155                 160                 165

att gac ctc gtaagtttcc ggcacgggcg tcgtcatcat cgagcggaaa             735
Ile Asp Leu

gactgaccgg ttaactgcag tac aat tat ggc tgc ccc cgg gtc ggt aac acc    788
                    Tyr Asn Tyr Gly Cys Pro Arg Val Gly Asn Thr
                        170                 175

gcg ctc gca gac ttc atc acc acg caa tcc gga ggc aca aat tac cgc      836
Ala Leu Ala Asp Phe Ile Thr Thr Gln Ser Gly Gly Thr Asn Tyr Arg
180                 185                 190                 195

gtc acg cat tcc gat gac cct gtc ccc aag ctg cct ccc agg agt ttt      884
Val Thr His Ser Asp Asp Pro Val Pro Lys Leu Pro Pro Arg Ser Phe
                200                 205                 210

gga tac agc caa ccg agc cca gag tac tgg atc acc tca ggg aac aat      932
Gly Tyr Ser Gln Pro Ser Pro Glu Tyr Trp Ile Thr Ser Gly Asn Asn
            215                 220                 225

gta act gtt caa ccg tcc gac atc gag gtc atc gaa ggc gtc gac tcc      980
Val Thr Val Gln Pro Ser Asp Ile Glu Val Ile Glu Gly Val Asp Ser
        230                 235                 240

act gca ggc aac gac ggc acc cct gct ggc ctt gac att gat gct cat     1028
Thr Ala Gly Asn Asp Gly Thr Pro Ala Gly Leu Asp Ile Asp Ala His
    245                 250                 255

cgg tgg tac ttt gga ccc att agc gca tgt tcg tga                    1064
Arg Trp Tyr Phe Gly Pro Ile Ser Ala Cys Ser
260                 265                 270
```

<210> 8

<211> 299

<212> PRT

<213> Talaromyces emersonii

<400> 8

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Met Phe Lys Ser Ala Ala Val Arg Ala Ile Ala Ala Leu Gly Leu Thr
                -25                 -20                 -15

Ala Ser Val Leu Ala Ala Pro Val Glu Leu Gly Arg Arg Asp Val Ser
            -10                 -5                  -1  1

Gln Asp Leu Phe Asp Gln Leu Asn Leu Phe Glu Gln Tyr Ser Ala Ala
    5                   10                  15
```

Ala Tyr Cys Ser Ala Asn Asn Glu Ala Ser Ala Gly Thr Ala Ile Ser
20 25 30 35

Cys Ser Ala Gly Asn Cys Pro Leu Val Gln Gln Ala Gly Ala Thr Ile
40 45 50

Leu Tyr Ser Phe Asn Asn Ile Gly Ser Gly Asp Val Thr Gly Phe Leu
55 60 65

Ala Leu Asp Ser Thr Asn Gln Leu Ile Val Leu Ser Phe Arg Gly Ser
70 75 80

Glu Thr Leu Glu Asn Trp Ile Ala Asp Leu Glu Ala Asp Leu Val Asp
85 90 95

Ala Ser Ala Ile Cys Ser Gly Cys Glu Ala His Asp Gly Phe Leu Ser
100 105 110 115

Ser Trp Asn Ser Val Ala Ser Thr Leu Thr Ser Lys Ile Ser Ser Ala
120 125 130

Val Asn Glu His Pro Ser Tyr Lys Leu Val Phe Thr Gly His Ser Leu
135 140 145

Gly Ala Ala Leu Ala Thr Leu Gly Ala Val Ser Leu Arg Glu Ser Gly
150 155 160

Tyr Asn Ile Asp Leu Tyr Asn Tyr Gly Cys Pro Arg Val Gly Asn Thr
165 170 175

Ala Leu Ala Asp Phe Ile Thr Thr Gln Ser Gly Gly Thr Asn Tyr Arg
180 185 190 195

Val Thr His Ser Asp Asp Pro Val Pro Lys Leu Pro Pro Arg Ser Phe
200 205 210

Gly Tyr Ser Gln Pro Ser Pro Glu Tyr Trp Ile Thr Ser Gly Asn Asn
215 220 225

Val Thr Val Gln Pro Ser Asp Ile Glu Val Ile Glu Gly Val Asp Ser
230 235 240

Thr Ala Gly Asn Asp Gly Thr Pro Ala Gly Leu Asp Ile Asp Ala His
245 250 255

Arg Trp Tyr Phe Gly Pro Ile Ser Ala Cys Ser
260 265 270

<210> 9

<211> 1074

<212> DNA

<213> Talaromyces byssochlamydoides

<220>

<221> CDS

<222> (1)..(85)

<223>

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<222> (150)..(318)

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<222> (376)..(709)

<223>

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<222> (760)..(1071)

<223>

<220>

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tca gtc ttt gct gct cct atc gaa ctg ggc cgt cga g gtaaggggca          95
Ser Val Phe Ala Ala Pro Ile Glu Leu Gly Arg Arg
        -10                 -5                  -1

tgaaaactcc ctgtatggca tctcatctgg cagcatatct actgacatcc tcag at       151
                                                          Asp
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gtt tcg gag cag ctc ttc aac cag ttc aat ctc ttc gag cag tat tcc      199
Val Ser Glu Gln Leu Phe Asn Gln Phe Asn Leu Phe Glu Gln Tyr Ser
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gcg gct gcg tac tgt cca gcc aac ttt gag tcc gct tcc ggc gcg gca      247
Ala Ala Ala Tyr Cys Pro Ala Asn Phe Glu Ser Ala Ser Gly Ala Ala
            20                  25                  30

att tct tgt tcc aca ggc aat tgc ccg ctc gtc caa cag gct ggc gca      295
Ile Ser Cys Ser Thr Gly Asn Cys Pro Leu Val Gln Gln Ala Gly Ala
        35                  40                  45

acc acc ctg tat gca ttc aac aa  gtgagtgtca tggaaaggct tgttggtaca     348
Thr Thr Leu Tyr Ala Phe Asn Asn
50                  55

ccgtacgggt atgttgactg tcatcag c atc ggc tct ggc gat gtg acg ggt      400
                              Ile Gly Ser Gly Asp Val Thr Gly
                                      60                  65

ttt ctt gct gtc gat ccg acc aac cga ctc atc gtc ttg tcg ttc cgg      448
Phe Leu Ala Val Asp Pro Thr Asn Arg Leu Ile Val Leu Ser Phe Arg
                70                  75                  80

ggg tca gag agt ctc gag aac tgg atc act aat ctc agc gcc gac ctg      496
Gly Ser Glu Ser Leu Glu Asn Trp Ile Thr Asn Leu Ser Ala Asp Leu
            85                  90                  95

gtc gat gcc tct gca atc tgt tcc ggg tgt gaa gcc cat gac gga ttc      544
Val Asp Ala Ser Ala Ile Cys Ser Gly Cys Glu Ala His Asp Gly Phe
        100                 105                 110

tat tcg tct tgg caa tca gtt gcc agc act ctg acc tcc caa atc tcg      592
Tyr Ser Ser Trp Gln Ser Val Ala Ser Thr Leu Thr Ser Gln Ile Ser
    115                 120                 125

tcg gcc ctc tcg gca tat cca aac tac aag ctg gtc ttc acc ggc cac      640
Ser Ala Leu Ser Ala Tyr Pro Asn Tyr Lys Leu Val Phe Thr Gly His
130                 135                 140                 145

agt ctc gga gcc gcc tta gct aca ctt gga gct gtc tct ctc agg gag      688
Ser Leu Gly Ala Ala Leu Ala Thr Leu Gly Ala Val Ser Leu Arg Glu
                150                 155                 160

agt gga tac aat atc gac ctc gtaagttcct ggcattgcca tcatggaaag         739
Ser Gly Tyr Asn Ile Asp Leu
            165

agactcacag ttaactgtag tac aac ttt ggc tgt ccc cgg gtc ggc aac act     792
                      Tyr Asn Phe Gly Cys Pro Arg Val Gly Asn Thr
                          170                 175

gcg ctc gca gac ttt att acc aac caa acc ggt ggc aca aat tac cgg      840
Ala Leu Ala Asp Phe Ile Thr Asn Gln Thr Gly Gly Thr Asn Tyr Arg
180                 185                 190                 195

gta acg cat tac gag gac cct gtc ccc aag ctg cct ccc agg agt ttt      888
Val Thr His Tyr Glu Asp Pro Val Pro Lys Leu Pro Pro Arg Ser Phe
                200                 205                 210

gga tac agc caa cct agc ccg gaa tac tgg atc acg tcg gga aac aat      936
Gly Tyr Ser Gln Pro Ser Pro Glu Tyr Trp Ile Thr Ser Gly Asn Asn
            215                 220                 225

gtg act gtg act tcg tcc gac atc gat gtc gtc gtg ggt gtc gac tcg      984
Val Thr Val Thr Ser Ser Asp Ile Asp Val Val Val Gly Val Asp Ser
        230                 235                 240
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act gca ggc aac gac ggg acg cct gat ggc ctt gac act gct gcc cat 1032
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Ser Val Phe Ala Ala Pro Ile Glu Leu Gly Arg Arg Asp Val Ser Glu
-10 -5 -1 1

Gln Leu Phe Asn Gln Phe Asn Leu Phe Glu Gln Tyr Ser Ala Ala Ala
5 10 15 20

Tyr Cys Pro Ala Asn Phe Glu Ser Ala Ser Gly Ala Ala Ile Ser Cys
25 30 35

Ser Thr Gly Asn Cys Pro Leu Val Gln Gln Ala Gly Ala Thr Thr Leu
40 45 50

Tyr Ala Phe Asn Asn Ile Gly Ser Gly Asp Val Thr Gly Phe Leu Ala
55 60 65

Val Asp Pro Thr Asn Arg Leu Ile Val Leu Ser Phe Arg Gly Ser Glu
70 75 80

Ser Leu Glu Asn Trp Ile Thr Asn Leu Ser Ala Asp Leu Val Asp Ala
85 90 95 100

Ser Ala Ile Cys Ser Gly Cys Glu Ala His Asp Gly Phe Tyr Ser Ser
105 110 115

Trp Gln Ser Val Ala Ser Thr Leu Thr Ser Gln Ile Ser Ser Ala Leu
120 125 130

Ser Ala Tyr Pro Asn Tyr Lys Leu Val Phe Thr Gly His Ser Leu Gly
135 140 145

Ala Ala Leu Ala Thr Leu Gly Ala Val Ser Leu Arg Glu Ser Gly Tyr
150 155 160

Asn Ile Asp Leu Tyr Asn Phe Gly Cys Pro Arg Val Gly Asn Thr Ala
165 170 175 180

Leu Ala Asp Phe Ile Thr Asn Gln Thr Gly Gly Thr Asn Tyr Arg Val
185 190 195

Thr His Tyr Glu Asp Pro Val Pro Lys Leu Pro Pro Arg Ser Phe Gly
200 205 210

Tyr Ser Gln Pro Ser Pro Glu Tyr Trp Ile Thr Ser Gly Asn Asn Val
215 220 225

Thr Val Thr Ser Ser Asp Ile Asp Val Val Val Gly Val Asp Ser Thr
230 235 240

Ala Gly Asn Asp Gly Thr Pro Asp Gly Leu Asp Thr Ala Ala His Arg
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Trp Tyr Phe Gly Pro Thr Thr Glu Cys Ser Ser Ser
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